PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL2005/000026	14.01.2005	16.01.2004

International Patent Classification (IPC) or both national classification and IPC
C12N5/08, C12N5/06, A61P17/02

Applicant
VERENIGING VOOR CHRISTELIJK WETENSCHAPPELIJK ...

1. This opinion contains indications relating to the following items:

- ☒ Box No I Basis of the opinion
- ☐ Box No II Priority
- ☒ Box No III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No VI Certain documents cited
- ☐ Box No VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66 1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later

For further options, see Form PCT/ISA/220

3 For further details, see notes to Form PCT/ISA/220

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NL2005/000026

Box No. I Basis of the opinion

1 With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item

☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material:

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material:

☐ in written format

☐ in computer readable form

c. time of filing/furnishing:

☐ contained in the international application as filed.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/NL2005/000026

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 20-24 (IA)

because:

☒ the said international application, or the said claims Nos. 20-24 (IA) relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Statement

Novelty (N)	Yes:	Claims	4, 10-13, 15-18
	No:	Claims	1-3, 5-9, 14, 19-24
Inventive step (IS)	Yes:	Claims	10, 11
	No:	Claims	1-9, 12-24
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	-

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/NL2005/000026

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 20-24 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 Basset-Séguin N et al., *Differentiation* September 1990, 44(3), 232-238
D2 Lee D-Y et al., *Journal of Dermatological Science* June 2000, 23(2), 132-137
D3 Chakrabarty K H et al., *Journal of Dermatology* November 1999, 141(5), 811-823
D3a Ghosh M M et al., *Annals of Plastic Surgery* October 1997, 39(4), 390-4
D4 Ralston D R et al., *Journal of Dermatology* April 1999, 140(4), 605-615
D5 Krejci N C et al., *Journal of Investigative Dermatology* November 1991, 97(5), 843-848
D6 Ponec M et al., *Skin Pharmacology & Applied Skin Physiology* Dec. 2002, 15(Suppl. 1), 4-17

Present claim 19 is a "product by process" claim. Under PCT Guidelines 5.26 and A5.26[1], which is relied upon by this Authority, the subject-matter of such a claim is required to possess absolute novelty, that is, novelty can only be acknowledged if the claimed product can be distinguished on its intrinsic characteristics from prior art products obtainable by any process.

D1 teaches a simplified method for preparing artificial skin for transplantation by growing skin punches biopsies, which comprises fibroblasts and keratinocytes from the subject, onto de-epidermised donor dermis (DED). The method of D1 differs from the method of present claims 1-18 in that fibroblasts and keratinocytes contact the same side of the DED connective layer. However, if fibroblasts from the punch biopsy infiltrate and populate the DED layer, the resulting reconstituted skin of D1, consisting of a DED layer populated by fibroblasts and an epidermal layer on top of the basement membrane attached to the DED, appears indistinguishable from the connective tissue substitute of claim 19 though it was formed in a different process. According to D3a (p. 398, fig. 3A), when fibroblasts and keratinocytes are cultured simultaneously on the papillary surface of DED, as occurs in the process of D1, fibroblasts do cross the basement membrane and grow within the DED. Therefore, the subject-matter of claims 19-24

is not novel in view of D1 (Article 33(2) PCT).

Similarly, D5 (p. 844) teaches the preparation of skin equivalents by cultivating fibroblasts and keratinocytes onto DED; keratinocytes, when used, are seeded in the same place, and thus on the same side, of the DED so that D5 does not anticipate the process of the application but once fibroblasts have infiltrated the DED, as is to be expected from the teachings of D3a, the resulting skin equivalent appears indistinguishable from the connective tissue substitute of claim 19 and therefore, the subject-matter of claims 19-24 is not novel in view of D5 (Article 33(2) PCT).

D2 teaches the preparation of a skin equivalent by contacting a sheet of DED with fibroblasts in a collagen matrix on one side and keratinocytes on the other side. In view of D2, the subject-matter of claims 1-3, 5-9, 14 and 19-24 is not novel (Article 33(2) PCT). Whether the cells and DED used in the process are autologous or heterologous (claims 12, 13, 15, 16, 18) are relative qualifications depending on the intended clinical use of the skin equivalent; besides the applicant acknowledges in its analysis of D2 (p. 3-4 of the present specification) that, given either extensive biopsies or extensive time to passage the cells, primary cells or autologous cells could be used in the process in D2 so the subject-matter of claims 4, 12, 13, 15, 16 and 18 pertains to readily available variants of the process and does not involve an inventive step (Article 33(3) PCT). Genetic engineering being well-known, neither does the additional subject-matter of claim 17 involve an inventive step over D2 (Article 33(3) PCT). While the process of the invention may actually have some advantages over D2 with respect to the biopsies required and the time required to grow a tissue substitute, these are not reflected in the present claims.

D3 (p. 814, referring back to D3a as ref. 10, for further details) D3a (p. 394, protocol 4) and D4 (p. 606) teach the preparation of skin equivalents by cultivating fibroblasts onto the reticular surface of DED for two days before cultivating keratinocytes onto the papillary surface. In view of either of D3, D3a or D4, the subject-matter of claims 1-3, 5-9, 14 and 19-24 is not novel and the subject-matter of claims 4, 12, 13 and 15-18 does not involve an inventive step (Article 33(2,3) PCT)—as for D2, given extensive biopsies, primary cells would be available instead of passaged cells, the auto- or heterologous nature of the materials is merely a relative qualification and genetic engineering could hardly contribute an inventive step. While the process of the invention may actually have some advantages over D3, D3a and D4 with respect to the biopsies required, the time and amount of manipulation required to grow a tissue substitute and the amount, these are not reflected in the present claims.

The subject-matter of claims 10 and 11 differs from D2-D4 in that an intact epithelial layer is used instead of isolated keratinocytes. The skilled person wanting to simplify the method of D2 would find in D1 some incentive for using pieces of whole tissues rather than cultivated cells, but D1 teaches the use of whole biopsies, comprising both an epidermal and a dermal layer, rather than of an epidermal layer dissociated from the dermal layer, as in the process of the invention. Thus the process of the

invention is not derivable from any of the prior art documents (D3 or D4 possibly being closest) even in combination with D1 and an inventive step can be acknowledged for the subject-matter of claims 10 and 11 (Article 33(3) PCT).

D6 is cited solely to illustrate that the particular culture medium used in the examples is not novel.

For the assessment of the present claims 20-24 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.